DRUGS TARGETED AT HIV – SUCCESSES AND RESISTANCE

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1. INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) was first identified as a previously unknown disease about twenty years ago, and since that time has become one of the most thoroughly studied human diseases. In the Western countries, it is most often caused by the human immunodeficiency virus type 1 (HIV-1), although in Africa it is commonly associated with another viral variant, HIV-2. The complete nucleotide sequence of HIV-1 was published in 1985 (Ratner *et al.*, 1985), and shows a relatively simple retrovirus, with the genome consisting of three open reading frames, *gag*, *pol*, and *env*. The *gag* open reading frame contains structural proteins such as capsid, nucleocapsid, and matrix, while regulatory proteins are encoded in the multiply-spliced *env* ORF. HIV-1 genome encodes only three unique enzymes, all of them within the *pol* open reading frame. They include reverse transcriptase (RT), a multi-domain enzyme involved in copying the RNA retroviral genome into DNA and in destroying the original RNA strand with its RNase H domain. Another enzyme is protease (PR), a symmetric homodimer related to cellular aspartic proteases such as pepsin, which is necessary for the cleavage of the viral polyproteins into individual mature proteins. Finally, HIV encodes viral integrase (IN), a multifunction processive enzyme which makes staggered cuts of the ends of viral DNA, inserting it in a non-specific manner into the host DNA.

The identification of the pathogen that causes AIDS required several years, and initially no drugs were available to fight the disease. However, since 1987, no fewer than 11 different anti-AIDS drugs have been approved for use in the United States (and in most other countries). Not surprisingly, all of these are inhibitors of the retroviral enzymes, RT and PR, although other drug design targets are still under active investigation and some novel potential drugs are now entering clinical trials. In this chapter, the design, properties, and the development of resistance against these drugs will be discussed.

The past 20 years have seen significant changes to the methodology of

pharmaceutical development (Hubbard, 1997). The complementary methods of computer-aided molecular design (Leach, 1996) and combinatorial chemistry (Balkenkohl *et al.,* 1996) are now routinely employed in both the lead identification and the compound development phases of drug design. Since these 20 years have coincided with the emergence of the AIDS pandemic, it is hardly surprising that the complete battery of techniques available to the pharmaceutical scientist have been unleashed on the HIV virus. The development of the protease inhibitors, in particular, owes a lot to the use of rational drug design techniques (Sansom, 1998; Vacca and Condra, 1997; Wlodawer and Vondrasek, 1998).

2. DRUGS TARGETING REVERSE TRANSCRIPTASE

The reverse transcriptase is a heterodimer consisting of two chains, p66 and p51, encoded by the same gene belonging to the *pol* ORF. The only difference in primary structure between these chains is that p51 does not contain the RNase H domain, which is found in p66 only. Although the amino acid sequences of the common parts of the two domains are identical, only p66 contains the active sites for both primary activities of the enzyme (RNA- or DNA-dependent DNA polymerase, and RNase H), while p51 is enzymatically inactive and is presumed to play a structural role only. The structures of RT have been solved in the presence of non-nucleoside inhibitors (see below) (Kohlstaedt *et al*., 1992); for the apoenzyme (Jager *et al*., 1994); in a ternary complex with a bound template:primer and Fab (Jacobo-Molina *et al*., 1993); and as a covalently trapped catalytic complex with a DNA template:primer and a deoxynucleoside triophosphate (Huang *et al*., 1998), among others. The structure of each domain (Figure 1) can be described as a right hand, with four domains denoted as fingers, palm, thumb, and connection (Kohlstaedt *et al*., 1992). The residues responsible for the polymerase activity include three aspartates in the palm domain (D110, D185, and D186), which is a counterpart of similar domains in other DNA and RNA polymerases. These aspartate residues act through bound divalent cations, which under physiological conditions consist of a pair of Mg^{2+} ions.

Reverse transcriptase inhibitors can be divided into two general classes. The first class to be discovered are compounds that act as terminators of chain elongation, that is, that can be incorporated by the enzyme into the newly synthesized strand, but which lack groups necessary for further extension of the chain. Chain terminators, which are analogs of the nucleoside substrates and bind in the substrate binding site, can inhibit both HIV-1 and HIV-2 RT, or any other polymerase that uses similar substrates. For these reasons, many were originally developed as cancer drugs, even though none of them found practical use for that purpose. Another class of RT inhibitors are compounds called non-nucleoside inhibitors (NNI's) that are specific to a pocket which is found in the vicinity of the active site in HIV-1 RT, but which does not exist in HIV-2 RT. These are noncompetitive inhibitors that were found largely by serendipity, and their practical application as AIDS drugs was originally subject to considerable controversy. Eventually some NNI's have been

found to be excellent drugs if used in combination therapy, even though they are largely useless in monotherapy.

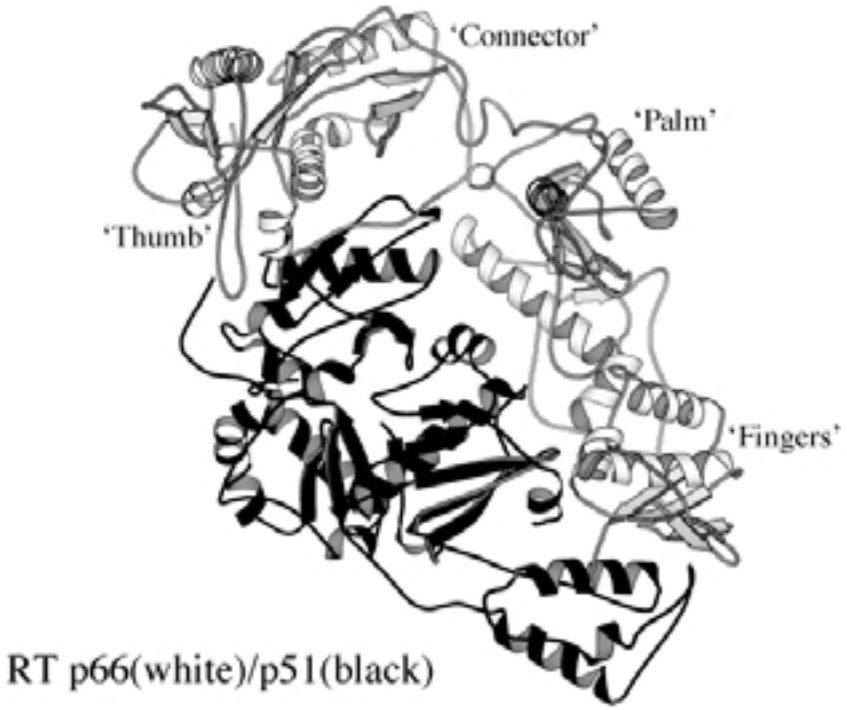

Figure 1. Ribbon structure of RT. The coordinates used to generate this figure are from Ren *et al.* (1995); PDB code 1REV. The active p66 domain is shown in gray, while the p51 domain is black.

2.1 Nucleoside Analogs

The nucleoside analogs are competitive inhibitors of HIV reverse transcriptase activity, even though they are not traditional mechanism-based inhibitors which bind to the enzyme tighter than the substrate (as are protease inhibitors, discussed below), but rather become incorporated into the growing product chains and prevent their further extension. Their inhibitory properties are due either to the lack of 2' or 3' hydroxyl groups, or to their replacement by other functional groups. In the case of AZT (azidothymidine, zidovudine), for example, the presence of the 3'-azido group prevents subsequent creation of a 3'-5' phosphodiester bond and thus terminates the chain (Figure 2).

The introduction of nucleoside analogs as potential AIDS drugs was based on the quickly growing understanding of the mechanism of action of retroviral enzymes such as RT, rather than on any detailed knowledge of their structure, which

was still unknown at the time when these compounds were introduced into medical practice. Nucleoside analogs are prodrugs in the sense that they need to be phosphorylated in order to gain inhibitory properties against RT. Such phosphorylation depends on the presence of specific enzymes in the host organism.

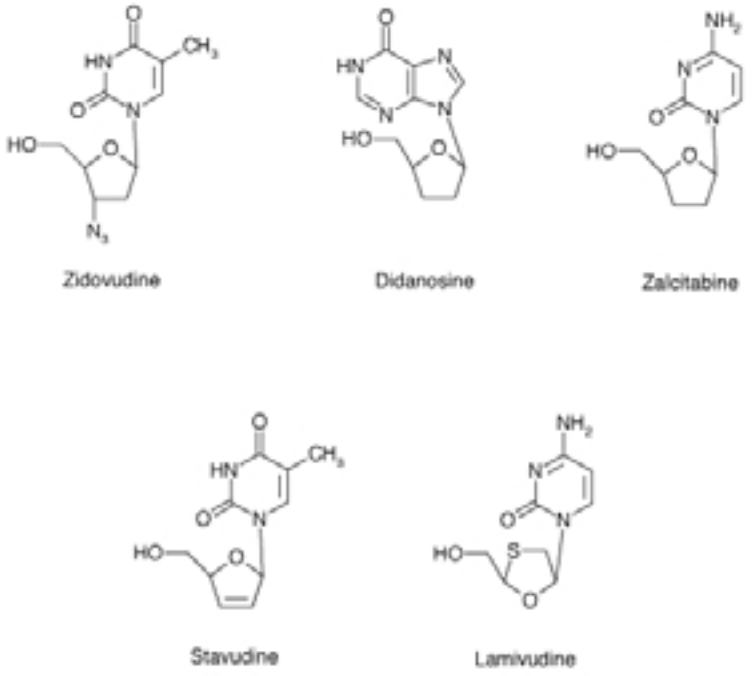

Figure 2. Chemical structures of clinically important nucleoside analog inhibitors of RT.

Five nucleoside analogs (NRTI's) have so far been approved by the U.S. Food and Drug Administration (FDA). The first of these was zidovudine (Retrovir, Glaxo-Wellcome). Its use was initially approved as a monotherapy in 1987, although its efficacy in that mode was shown to be only transitory (Volberding *et al*., 1990). However, the importance of zidovudine as a therapeutic agent against AIDS cannot be overemphasized, since for several years it was the only generally available approved drug. The activities of all other anti-HIV compounds have been routinely compared to it ever since. In particular, usefulness in preventing transmission of HIV from mother to child during pregnancy was of special note. The drug is delivered orally and has very high bioavailability in that mode. Its affinity for HIV-1 RT is two orders of magnitude higher than for human DNA polymerase, thus minimizing potential side effects, which are, however, still considerable. The most

common side effect of the continuous use of zidovudine is anemia.

Another nucleoside analog AIDS drug is didanosine (dideoxyinosine: ddI, Videx), a product of Bristol-Myers Squibb. It is an analog of inosine, lacking both the 2'- and 3'-hydroxyl groups on its ribose moiety. In common with zidovudine, its active form is also a triphosphate, produced by a cellular enzyme. Its intracellular half-life is 8-24 hours, much longer than that of zidovudine, thus allowing once-a-day dosing. Since the compound is inactivated by an acidic environment, it is usually buffered in order to increase gastric pH.

Another drug related to didanosine is zalcitabine (dideoxycytidine: ddC, Hivid), a product of Hoffmann-La Roche. This compound has been FDA-approved since 1992 and its mode of action is the same as that of other nucleoside analogs. This pyrimidine analog is active against HIV in vitro at very low concentrations, although its plasma half-life is rather short, requiring several daily doses of the drug. The principal side effects are pancreatitis and peripheral neuropathy.

Stavudine (d4T: 3'-deoxy-2'-thymidinene, Zerit), manufactured by Bristol-Myers Squibb, is a modification of thymidine. It has been used in clinical practice since 1993, and was approved for the treatment of HIV-infected adults who have received prolonged zidovudine therapy (Spruance *et al*., 1997). The drug has been found to be generally well tolerated and has minimal side effects.

The latest of the currently approved nucleoside inhibitors of RT is lamivudine (3TC: 3'-thio-2',3'-dideoxcytidine, Epivir), which was discovered by Bio-Chem Pharma and developed by Glaxo-Wellcome. It gained FDA approval in 1995. In addition to being a potent AIDS drug, lamivudine has also been shown to be potent against another viral disease, chronic hepatitis B. Since lamivudine has been shown to elicit very rapid resistance (see below), its initial development was questionable. However, after it was found that the principal mutation due to the exposure to lamivudine, M184V, prevents resistance to zidovudine, a combination of both drugs (Combivir, which consists of 150 mg of lamivudine combined with 300 mg of zidovudine) was developed.

2.2 Non-nucleoside analogs (NNI's)

All members of this heterogeneous class of compounds (Figure 3) are potent inhibitors of HIV-1 RT (but not of HIV-2 RT, or indeed of reverse transcriptase from any other retroviruses). The first few of these compounds, such as HEPT (Baba *et al.*, 1989) or TIBO (Pauwels *et al*., 1990) were discovered to be active in cell culture before their target was identified. A number of other members of this class, including nevirapine, were identified in screening programs specifically targeting HIV-1 RT (Merluzzi *et al*., 1990). The NNI's bind in a pocket located about 10 Å from the substrate-binding site, which includes residues such as Val179, Tyr181, Tyr188, and Trp229. When bound into that pocket in HIV-1 RT, most NNI's maintain a similar, butterfly-like shape. Most of them superimpose structurally quite well and appear to act as -electron donors to the aromatic side chains surrounding the pocket (Kroeger *et al*., 1997). The mode of action of NNI's is not completely clear, although it has been suggested that they might alter the conformation of the active

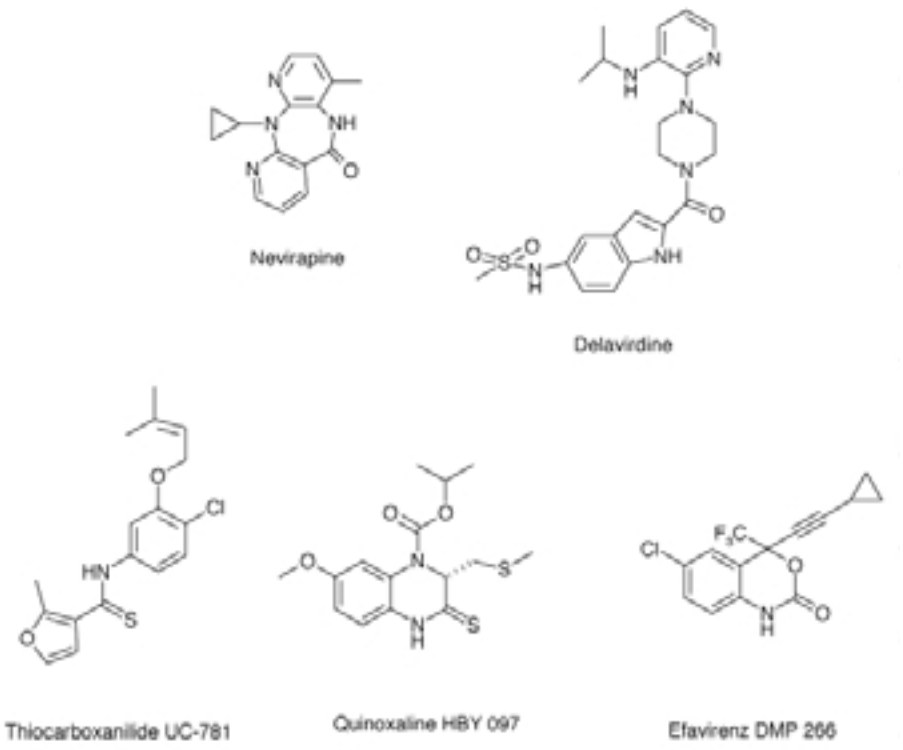

Figure 3. Chemical structures of clinically important non-nucleoside RT inhibitors.

site due to their proximity, or else restrict the motions of the p66 thumb domain (Kohlstaedt *et al*., 1992).

So far, two NNI's have gained FDA approval for use against HIV (De Clercq, 1998). Nevirapine (Viramune, manufactured by Boehringer-Ingelheim) was the first of these to gain approval in 1996. This compound is highly bioavailable in oral form. It induces its own metabolism by activating the hepatic cytochrome P450 pathways. The main side effect of its utilization is a rash. The structure of a complex of nevirapine with RT was the first reverse transcriptase structure to be determined by x-ray crystallography (Kohlstaedt *et al*., 1992).

Another FDA-approved NNI is delaviridine (Rescriptor), manufactured by Pharmacia and Upjohn. This compound is usually prescribed in combination with other anti-HIV drugs, either nucleoside RT inhibitors or PR inhibitors. It has relatively short plasma half-life, requiring several daily doses to maintain its concentration.

2.3 Development of resistance to drugs targeting RT

Due to the lack of an editing function in retroviral RT, transcription errors during nucleic acid replication are very common, and the viral pool contains species with all conceivable mutations. The presence of drugs provides a powerful selection pressure for virus modifications that produce lower susceptibility to such compounds. This is especially true for monotherapy, in which a single drug is expected to provide suppression of viral replication. Development of resistance is often observed very soon after initiation of therapy, often after only a week or two. Such phenomena were observed early on, when zidovudine was the only approved AIDS drug, and rapid appearance of drug-resistant HIV species was considered a major block in the development of newer therapies, such as PR inhibitors or NNI's.

While nucleoside inhibitors of RT have been in clinical use for almost 15 years, the mechanism of resistance to them has been elucidated only recently in the studies by Huang and colleagues (1998). The structure of a trapped catalytic complex of RT provided data on the exact location of the incoming deoxynucleoside triphosphate and, by extension, of the nucleoside drugs. Not surprisingly, the point mutations sufficient for resistance (K65R, K70R, L74V, Q151M, M184I/V, and T215Y) are all located in the vicinity of the incoming nucleotide and may affect directly the position, stability, or reactivity of the bound analog (Figures 4a, 5a). There appears to be direct correlation between the location of mutation sites and the chemical nature of the analogs, explaining why mutations such as K70R are particularly responsible for the development of resistance to zidovudine with its extra azido group. This mutation is often followed by further ones such as T215Y/F, K210W, which may stabilize the mutation of residue 215, M41L, D67N, and K219Q. Conversely, mutations responsible for the resistance to dideoxynucleotides are located on the opposite surface of the enzyme and include L74V, M174V, K65R, and T69D. The mutation Q151M arises primarily in the patients on dual therapy (zidovudine and didanosine or zalcitabine), affecting both contact with the deoxynucleoside triphosphate and the character of the 3' pocket. An analysis of the steric nature of these mutations can also explain why resistance to one class of inhibitors may sensitize the enzyme to another class, forming the basis of sequential therapy.

The emergence of resistance to NNI's is particularly rapid, since the binding site for these compounds in not a direct part of the active site of RT (9, Schinazi *et al.*, 1997). As a rule, mutations leading to resistance to NNRTI's involve residues lining the binding site of these inhibitors (Figures 4b, 5b). Two mutations, K103N and Y181C, are induced by almost all known NNRTI's except the quinoxalines (De Clercq, 1998). Other common mutations which induce resistance to one or more NNRTI's include L100I, V106A/I/L and G190A/T/V. It was originally thought that the rapid emergence of resistant variance would compromise the utility of these drugs in the clinic. However, it is now clear that resistance can be minimized, both by using NNRTI's in combination with other inhibitors and by starting therapy with high concentrations of the drugs. NNRTI's are generally prescribed in combination with drugs from other classes (Coleman and Holtzer, 1998).

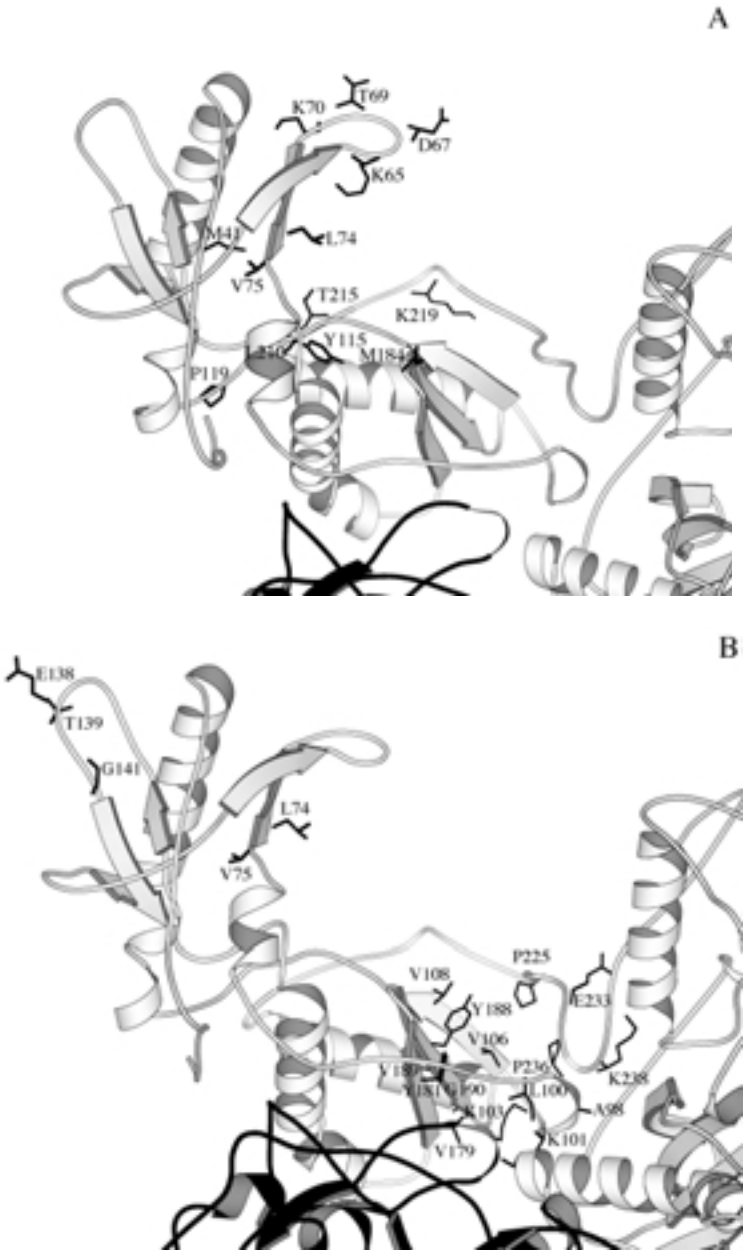

Figure 4. Ribbon structure of a fragment of RT showing amino acids with mutations arising from the use of either **A:** NRTI's or **B:** NNRTI's.

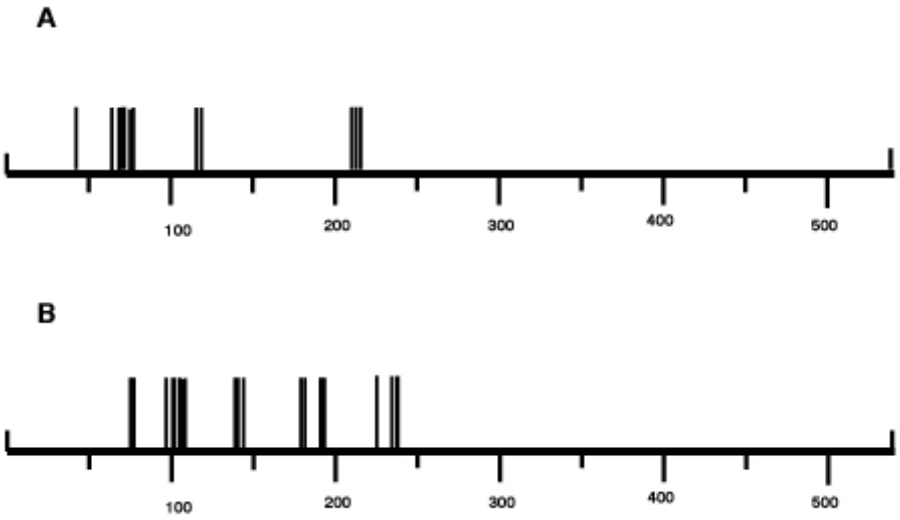

Figure 5. Schematic diagram comparing positions of RT mutations arising from the use of NRTI's and NNRTI's. Data taken from Schinazi *et al*. (1997). **A**: mutations arising from the use of NRTI's; **B**: mutations arising from the use of NNRTI's.

3. DRUGS TARGETING PROTEASE

Retroviral proteases are unique proteins in the sense that they are the only known homodimeric enzymes containing a single, symmetric active site, which includes two adjacent aspartic acid side chains (Figure 6). One aspartate is protonated and the complex is stabilized by a hydrogen bond between them (Pearl, 1987). While each of the two molecules forming a retroviral protease resembles a domain of a single-chain aspartic protease such as pepsin or renin, the latter enzymes retain only approximate twofold symmetry. However, the similarity of the retroviral protease to cellular aspartic proteases was invoked early on as the reason to concentrate on designing inhibitors of that enzyme as potential AIDS drugs. There was a long history of attempts to design renin inhibitors as antihypertensive drugs (Greenlee, 1990). While no such drugs have ever been successfully introduced, the immense experience gained in that work turned out to be invaluable for designing pharmacologically successful inhibitors of HIV PR.

In both HIV-1 and HIV-2 PR, each chain consists of 99 amino acids, with about 50% identity between the enzymes from these two variants of HIV. The principal active site residue is Asp25. Mutation of this residue to any other, including Asn, completely inactivates the enzyme (Kohl *et al*., 1988). The determination of the crystal structure of the apoenzyme (Navia *et al*., 1989; Wlodawer *et al*., 1989) was rapidly followed by the structure of a complex with a substrate-based inhibitor (Miller *et al*., 1989), with many other similar structures to follow (Wlodawer and Erickson, 1993; Wlodawer and Vondrasek, 1998). These structures, as well as a

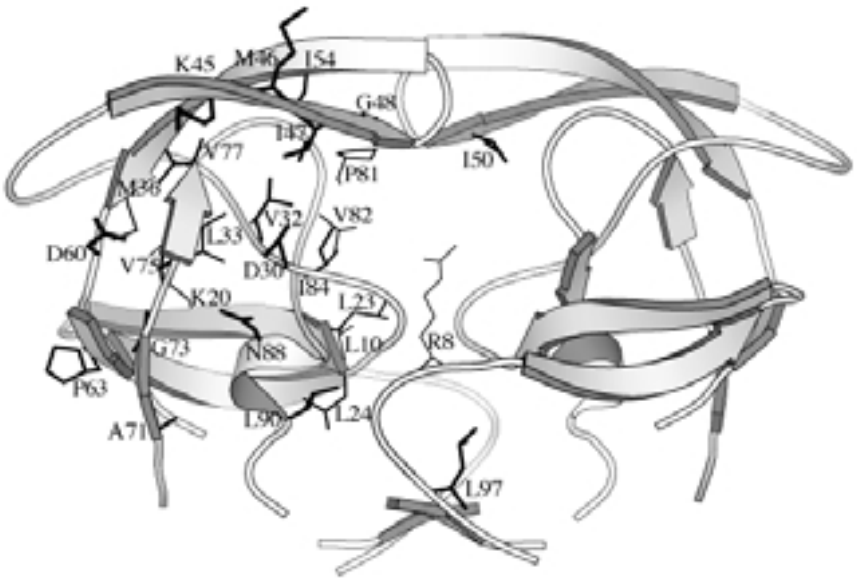

Figure 6. Ribbon structure of protease showing amino acids with drug-resistant mutations found in one of the two identical subunits. Structure based on Swain *et al.* (Swain *et al.*, 1990); PDB code 7HVP.

number of structures solved using NMR (Yamazaki *et al*., 1996) provided crucial information for the design of new classes of inhibitors, which, ultimately, became an important new category of AIDS drugs (Vacca and Condra, 1997; Wlodawer and Vondrasek, 1998). All publicly available structures of HIV PR and the related simian retrovirus SIV have been made available on the Web in the HIVdb database (Vondrasek and Wlodawer, 1997; Wlodawer and Vondrasek, 1999). The current version of HIVdb, released in early 1999, contains 111 different structures of HIV-1 PR, of which 27 (24%) are mutants, and 23 structures of HIV-2 PR of which 12 (52%) are mutants. The database contains structures of HIV-1 PR in complex with all approved protease drugs, and many other potential drugs and drugs in development; over 35% are "unofficial releases" of structures not available in the Protein Data Bank. This is a useful resource for studying the structural effects of mutations and their influence on drug interactions.

3.1 Drugs interacting with the active site

A very large number of compounds aimed at inhibiting HIV PR by binding to the active site of the enzyme have been created (Figure 7). The initial approach was based on the observation that the smallest oligopeptide substrates that can be efficiently processed by the enzyme consist of about six amino acids, three on the N-terminal side and three on the C-terminal side of the bond to be cleaved. Each

amino acid of the substrate occupies a matching subsite in the enzymes, with the subsites on both sides of the scissile bond being symmetric. The early inhibitors were peptide-mimetics: peptides mimicking the specific protease cleavage sites, with a nonscissile insert substituting for the peptide bond which would be normally cleaved. A particular sequence, cleaved effectively by retroviral proteases only, but not by the related cellular enzymes, contained a central sequence Tyr-Pro or its modifications. This dipeptide was used in the design of many early inhibitors.

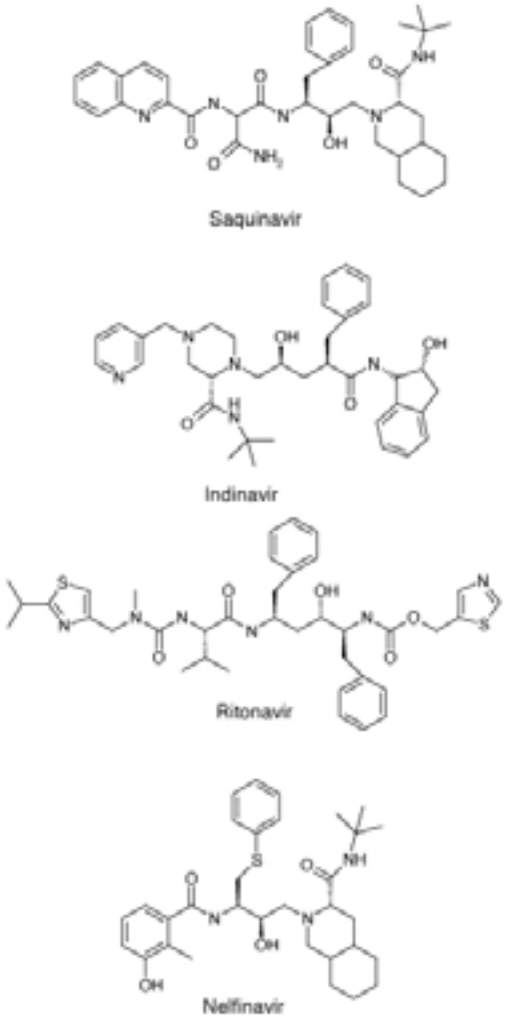

Figure 7. Chemical structures of clinically important protease inhibitors.

Four PR inhibitors have gained FDA approval as AIDS drugs. The first of these, saquinavir (Ro 31-8959; initially formulated by Hoffmann-La Roche as hard

gel capsules under the name of invirase, and later as soft gel capsules as fortovase) was approved in 1995. This compound was created from a short peptide by replacing the scissile peptide bond by a hydroxyethylamine moiety, unexpectedly with *R* stereochemistry. The proline residue that followed the nonscissile insert was changed to (S, S, S)-decahydroisoquinoline-3-carbonyl (DIQ), resulting in a considerable increase in potency. While the presence of several chiral centers made the synthesis difficult, and its bioavailability was rather poor, saquinavir turned out to be quite useful, especially due to its almost negligible side effects.

Ritonavir (Norvir) was developed at Abbott Laboratories in a program that initially made significant use of the symmetric nature of HIV PR, as compared with the only quasi-symmetric nature of cellular proteases. This compound also utilizes a hydroxyethylene replacement for the scissile bond, located between two phenylalanines, but in *S* stereochemistry. Unexpectedly, ritonavir was found to be a powerful inhibitor and inducer of the cytochrome P450 metabolic pathway system, and thus the initial dose of the drug needs to be subsequently raised to maintain its proper level. This drug is the least well-tolerated of all the approved protease inhibitors, but its side effects (related to its P450 activity) can be made its actual strength, by utilizing it together with new-generation inhibitors such as ABT-378, which is now undergoing Phase II clinical trials (Carillo *et al*., 1998). This unexpected property of ritonavir may be the main reason for its use in the future.

Indinavir (Crixivan), developed by Merck Pharmaceuticals, was licensed by FDA in 1996. Like saquinavir, it was designed on the peptide-mimetic principle. The drug should be given only under fasting conditions, making its delivery comparatively more difficult. It is, however, relatively safe and well tolerated. In combination with other AIDS drugs such as zidovudine and lamivudine, indinavir was shown to be capable of durable suppression of the virus for periods as long as over 2 years.

Nelfinavir (Viracept) is an Agouron Pharmaceuticals drug, which was approved for adult use in 1997. Subsequently it became the first protease inhibitor to be approved for pediatric use. Chemically it shares parts of its structure (DIQ group) with saquinavir, although it is much smaller, and has much higher oral bioavailability. Since its introduction two years ago it has become the most highly prescribed PR inhibitor, even though it exhibits some side effects, particularly diarrhea.

Several protease inhibitors are in advanced clinical studies and thus widely available; the following list is almost certainly only partial. Of these, the Glaxo-Wellcome drug amprenavir (also known as 141W94, or under the designation VX-478 by Vertex, the company where it was first synthesized) is probably the closest to full approval. Because of its long half-life, it can be administered only twice a day. Pharmacia and Upjohn is conducting an early clinical trial of PNU-140690, a unique PR inhibitor with completely non-peptidic character. Triangle Pharmaceuticals is developing DMP-450, a symmetric cyclic urea derivative with unique properties, originally designed at DuPont Merck.

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3.2 Resistance to PR inhibitors

The appearance of HIV strains with reduced susceptibility to PR inhibitors has been monitored both *in vivo* and *in vitro*, using clinically approved inhibitors, as well as a variety of other ones. A recent study of such mutations has shown that about one third of the residues in HIV-1 PR have been found to be mutated in samples obtained with the help of 21 drugs (Schinazi *et al*., 1997) (Figures 6, 8). While some of these mutations are in the pocket directly adjacent to the inhibitors, other mutations are observed throughout the protein. The appearance of the mutations is usually sequential, and remote mutations usually develop subsequently to the primary ones.

The most common mutations elicited by saquinavir are G48V and L90M, both in the drug-binding pocket. These mutations decrease the potency of the inhibitor several-fold. The nature of the formulation of the drug (soft or hard capsules) does not modify the pattern of resistance. The discovery of a pattern of multiple resistance mutations in patients subjected to indinavir monotherapy (Condra *et al*., 1995), as well as cross-resistance with six other PR inhibitors, has raised serious questions about the possible efficacy of the drugs belonging to that category. This initial pessimism, however, has turned out to be unwarranted, since the use of sufficiently high doses of the drugs, and combination therapies, have been shown to be quite successful in delaying or overcoming the appearance of resistance.

The importance of the appearance of drug-resistant mutants of HIV PR is considered to be so high that resistance studies now precede any attempts of introducing such compounds into clinical practice. A good example is provided by AT-378, a new PR inhibitor from Abbott Laboratories, which is currently in phase II clinical trials. Serial passages of the virus grown in the presence of the inhibitor established a sequential pattern of resistance development The mutations appear in the order I84V, L10F, M46I, T91S, V32I, and I47V. Further selection led to a mutation V47A, followed by reversion of I32 back to V (Carillo *et al*., 1998). It is likely that similar mutations will also be observed *in vivo*. Clearly, the development of drug-resistant strains of HIV is quite complicated.

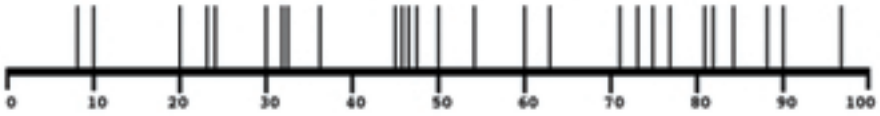

Figure 8. Schematic diagram showing mutations in PR arising from the use of clinically important PR inhibitors.

4. DRUGS TARGETING INTEGRASE

HIV integrase (IN) is the third enzyme found in the viral genome, and thus is a

natural target for drug design. This three-domain protein is absolutely required for the support of viral life cycle, with its role being the processing of the DNA copy of the viral genome and its integration into the genome of the host. While the structure of intact integrase is not yet available, crystal and NMR structures of individual domains have been published in the last few years (Cai *et al*., 1997; Dyda *et al*., 1994; Eijkelenboom *et al*., 1995; Eijkelenboom *et al*., 1997; Goldgur *et al*., 1998; Lodi *et al*., 1995; Maignan *et al*., 1998). These structures provided detailed data which is potentially very useful for drug design, although at this time no drugs have been approved, and only one clinical trial is under way (see below).

The amino- and carboxy-terminal domains of integrase are directly involved in the binding of the DNA substrates, but not directly in the catalytic activity of the enzyme. The central domain (residues ~50-210) (Figure 9) is directly responsible for the catalysis by integrase, and is capable of at least limited catalytic activity in the absence of the two other domains. The structure of that domain (Dyda *et al*., 1994) elucidated a close relationship between integrase and other nucleotidyl transferases such as RNase H, phage μ resolvase, and RuvC. The enzyme also bears distant relationship to a number of DNA and RNA polymerases. It was puzzling, however, that the active site as seen in the structure did not show a typical divalent cation binding motif, especially since the crystal structure of a related integrase from avian sarcoma virus showed that it could bind divalent cations such as magnesium or manganese (Bujacz *et al*., 1996). This discrepancy was recently resolved with new structures of the catalytic domain of HIV IN, which showed bound cations and proved that the original structure corresponded to an inactive enzyme (Goldgur *et al*., 1998; Maignan *et al*., 1998).

Although extensive efforts to discover inhibitors of integrase have been going on for a number of years (Pommier *et al*., 1997), the only putative integrase inhibitor currently under clinical development is AR-177 (Zintevir) from Aronex. That compound belongs to the family of guanosine-quartets but while it can interact with integrase (Cherepanov *et al*., 1997), it might inhibit HIV primarily by a completely different mechanism, involving interactions with the envelope glycoprotein gp120 (Este *et al*., 1998). Neither the future therapeutic potential of Zintevir nor its mode of action are certain at this time. Interestingly, one of the indications that integrase might not be its primary target comes from an in vitro study of the resistant viral strains, which showed mutations in the gp120 gene, but no mutations in the part of the genome encoding integrase.

5. STRATEGIES TO OVERCOME DRUG RESISTANCE

In the decade and a half since the first anti-HIV drug entered clinical trials, it became absolutely clear that any monotherapy utilizing enzyme inhibitors must lead to the emergence of resistant strains of the virus. While the time until such resistance is apparent varies among different classes of drugs and the individual compounds, it may be extremely rapid. It can be as short as about a week for a non-nucleoside analog nevirapine (Coleman and Holtzer, 1998). Combination therapies promise a

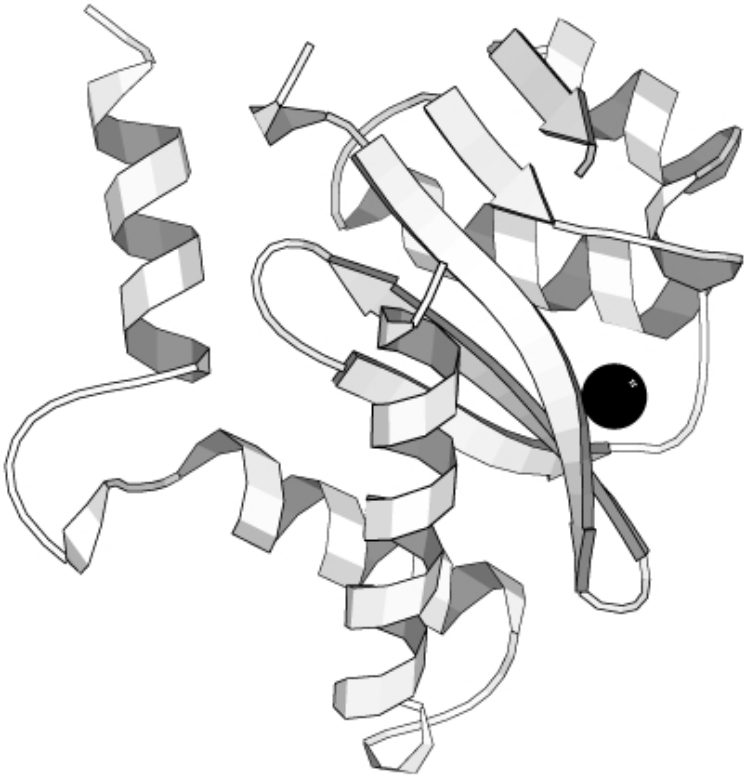

Figure 9. Ribbon structure of the central catalytic domain of integrase, based on the structure by Goldgur *et al*. (1998); PDB code 1BIU. The ball corresponds to a Mg^{2+} ion.

much better outcome, although there is no clear agreement at this time on which particular drug combinations might be the best. In particular, it is not clear whether it is better to use combinations of different drugs from the same family, or drugs belonging to different classes. On one hand, combinations such as ritonavir-saquinavir, nelfinavir-saquinavir, or ritonavir-indinavir combine two similar drugs but of distinct resistance pattern and, especially in the case of ritonavir, of different metabolism. On the other hand, combinations of indinavir or nelfinavir with nevirapine, or indinavir plus efavirenz, assure that the development of resistance will require mutations in two different enzymes, making such events less likely. In any case, starting from the initial clinical trials most therapeutic regimens utilizing protease inhibitors and non-nucleoside RT inhibitors also utilized nucleosides as backup. Since the combinations of nucleoside and non-nucleoside drugs are often effective, they are sometimes preferred in order to leave PR inhibitors as potential backup (Coleman and Holtzer, 1998) if the RT therapy fails.

In Western countries, drug treatment is reducing AIDS to a manageable

and treatable long-term disease. However, even with eleven drugs already on the market, it is clear that the serious nature of the AIDS pandemic and the limitations of the therapies make it necessary to continue the process of drug development. Until a safe, effective vaccine against HIV has been developed, it will always be necessary to introduce new therapies and combinations of drugs to counteract the development of resistant variants. It is also important to consider expense, as the current drugs cost thousands of dollars a year per patient. Any drug that is to be of use in those Third World countries where AIDS is most prevalent must be cheap and easy to synthesize. It may also be necessary to develop drugs specifically targeted to HIV-2 to counteract the African pandemic. The understanding of the drug-target interactions on the molecular levels, coupled with extensive studies using the techniques of molecular biology, are of great help in achieving rapid success of this work.

ACKNOWLEDGEMENTS

Research sponsored in part by the National Cancer Institute, DHHS, under contract with ABL. We thank Jerry Alexandratos for help in preparation of the figures. A.W. would like to thank the Master and Fellows of the Sidney Sussex College and the Department of Biochemistry, University of Cambridge, for a Visiting Fellowship during which tenure this paper was written. The contents of this publication do not necessarily reflect the views or policies of the Department of Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

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